

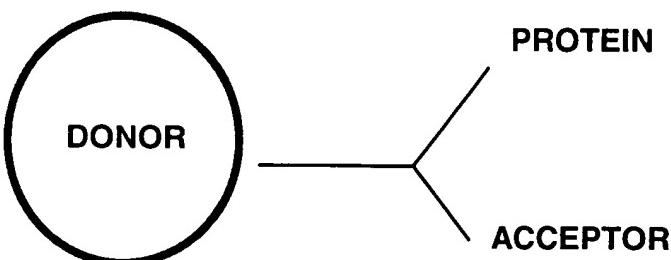
# CETP Activity Kit

## Cholesteryl Ester Transfer Protein Activity Kit

**Overview** - The cholesteryl ester transfer protein (CETP) is a protein present in normal human plasma. The CETP transfers neutral lipids from high density lipoprotein (HDL) to very low density lipoprotein (VLDL). The CETP Activity Kit includes donor and acceptor particles. Incubation of donor and acceptor with CETP source results in the CETP mediated transfer of fluorescent neutral lipid. The fluorescent neutral lipid is present in a self-quenched state when contained within the core of the donor. The CETP mediated transfer is determined by the increase in fluorescence intensity as the fluorescent neutral lipid is removed from the self-quenched donor to the acceptor.

**FOR RESEARCH USE ONLY**

**COMBINE:**



**METHOD**

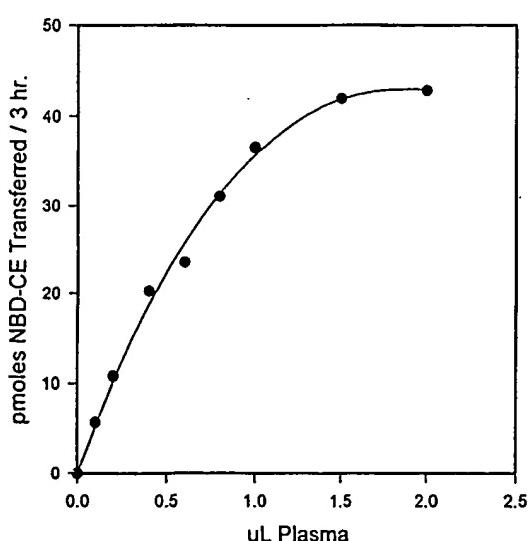
- Combine 10  $\mu$ l donor and 10  $\mu$ l acceptor with CETP source (1-3  $\mu$ l plasma or serum - fresh or frozen) in 0.5 ml total volume of buffer (10 mM tris, 150 mM NaCl, 2 mM EDTA) at pH 7.4
- Incubate for 3 hours at 37 degrees centigrade
- Read assay in a fluorescence spectrometer at excitation wavelength of 465 nm and emission wavelength of 535 nm

**INCUBATE:** 37°

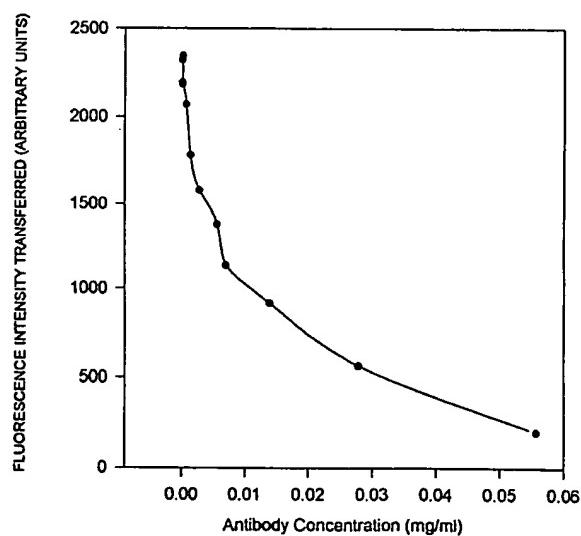
Activity is Assessed as an Increase in Fluorescence

**READ:**

**CETP Activity in Human Plasma**



**Inhibition of NBD-CE Transfer by an  $\alpha$ -CETP Ab**



**NOTES:** 50 mM tris interferes with the assay. Fluorescent assays are highly sensitive and will respond to slight changes in assay volume -- **BE SURE TO CAP TUBES** -- microplate incubations must be placed in sealed container with standing water to prevent evaporation. If plasma is used as CETP source, transfer will still occur without exogenous acceptor due to endogenous plasma lipoproteins. Results from duplicates should be tight. Variability indicates evaporation, inaccurate pipetting or incomplete mixing of assay components.

Donor and Acceptor may be mixed with the buffer and pipetted as one step. Plasma should be diluted ten-fold and then pipetted.

Microplate incubations must be able to raise temperature of assay to 37°C rapidly. Test your incubator with a small container of water and a thermometer. Large, humidified air incubators have caused problems by slowly increasing temperature from 25° to only 34° after three hours. Tubes should be incubated in a water bath type incubator.

Microplates must be compatible with fluorescent assays. Some clear plates contain fluorescent plastic.

The filters must be within specification. An excitation filter of 485 nm with a 20 nm bandwidth may **NOT** be used. This filter will incompletely excite the label and the standard curve will appear to work but your protein activity results will be low.

#### **RECOMMENDED PLATES**

- Millipore CytoPlate low fluorescence plastic #CFCP N96
- Dynex Laboratories black plates (must be used only with top reading plate readers)

#### **Ordering Information**

#RB-CETP      CETP Activity Kit \*100 assays\*

#### **Contact Information**

**Roar Biomedical, Inc.**  
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Audubon Biomedical Center  
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New York, NY 10032 USA  
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#### **Standardization**

The batch number of the kit and concentration of the fluorescent substrate is printed on the label.

The standard curve to derive the relationship between fluorescence intensity and mass transfer is generated by dispersing donor particle in isopropanol. Spectrally pure (HPLC grade or better) isopropanol is utilized as the solvent. There should be no background fluorescence when isopropanol alone is read at EX 465 nm / EM 535 nm.

Prepare six test tubes labeled from 'T0' to 'T5' each containing 1 ml isopropanol, add an additional 1 ml of isopropanol to 'T5'.

Pipette 5 µl donor particle to the test tube labeled 'T5', thoroughly mix (vortex) to adequately disperse the donor particle in the isopropanol.

Transfer 1 ml 'T5' (concentration = (X moles/ml x (0.005)/2) / ml isopropanol) to the test tube labeled 'T4'. Mix and pipette 1 ml from tube 'T4' to tube 'T3', vortex tube 'T3'. Pipette 1 ml from tube 'T3' to tube 'T2', vortex tube 'T2'. Pipette 1 ml from tube 'T2' to tube 'T1', vortex.

Read the fluorescence intensity (EX 465 / EM 535) of the samples from tubes 'T0' to 'T5'.

Apply the fluorescence intensity values of the standard curve directly to your results to express specific activity of the plasma samples.